IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Hiroyuki Tsunoda *et al.*	Art Unit	: 1633
Serial No.	: 10/581,183	Examiner	: Fereydoun Ghotb Sajjadi
Filed	: May 18, 2007	Conf. No.	: 1638
Title	: EXPRESSION SYSTEMS USING MAMMALIAN BETA-ACTIN PROMOTER		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Hiroyuki Tsunoda Under 37 C.F.R. § 1.132

I, the undersigned, Hiroyuki Tsunoda, residing at 153-2 Nagai, Niihari-mura, Niihari-gun, Ibaraki, JAPAN, declare and state as follows:

1. I am a co-inventor named on U.S. Patent Application No. 10/581,183 ("the Application").

2. My curriculum vitae is attached as Exhibit A.

3. I have reviewed and am familiar with the Office Action dated August 14, 2009 ("the Office Action"), issued by the U.S. Patent and Trademark Office in the Application.

4. I have reviewed and am familiar with the Application's specification, filed on May 18, 2007, and currently pending claims.

5. Exhibit B is a graph illustrating the results of an experiment that was conducted under my direction and control, using the experimental procedures generally described in Example 2 of the Application. In this experiment, the activities of various promoters (mouse β actin promoter, chicken β actin promoter, and human EF1α promoter), alone or in combination with human CMV enhancer and in one case WPRE, were compared using a luciferase reporter gene assay. Specifically, the promoter (with or without human CMV and/or WPRE) was cloned into the multiple cloning site of pGL3-Basic (Promega, Cat. No. E1751), and this vector was transiently expressed in DG44 cells. Luciferase activity was determined using the Luciferase Assay System (Promega, Cat. No. E1501). After removing the medium, 100 µl of passive lysis buffer was added to each well, and the cells were lysed by shaking. 10 µl of the cell lysate was transferred to Assay Plate Tissue Culture Treated White with Clear Bottom (Corning, Cat No. 437842). The measurement was performed using MicroLuMAT (Berthold), and the data were collected using the WinGlow-Control Program LB96PV software, ver.1.24.

6. As shown in Exhibit B, the combination of a mouse β-actin promoter and a human CMV enhancer ("CMV-mAct") drives a significantly higher level of luciferase reporter gene expression as compared to a conventional DNA construct utilizing a chicken β-actin promoter and a human CMV enhancer ("CAG"). Additionally, the combination of a mouse β-actin promoter, a human CMV enhancer, and a WPRE enhancer ("CMV-mAct-WPRE") drives a significantly higher level of luciferase reporter gene expression as compared to the conventional DNA construct.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Hiroyuki Tsunoda.

Hiroyuki Tsunoda

Date: February 15, 2010